

What is claimed is:

1. A method of determining disease status of a patient suffering from a disease characterized by aberrant expression of one or more intracellular complexes, the method comprising the steps of:

5 measuring directly in a patient sample an amount of each of one or more intracellular complexes;

comparing each such amount to its corresponding amount in a reference sample; and

correlating differences in the amounts from the patient sample and the respective corresponding amounts from the reference sample to the disease status the patient.

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2. The method of claim 1 wherein said patient sample is a fixed tissue sample, a frozen tissue sample, or circulating epithelial cells.

3. The method of claim 2 wherein said one or more intracellular complexes are selected from the group consisting of 14-3-3//BAD, BID//BAX, BAX//BAX, Bcl-X<sub>L</sub>//BAD, Bcl-2//BAD, 14-3-3//BID, BID//BAK, BAX//Bcl-2, Bcl-X<sub>L</sub>//BIK, Bcl-2//BIK, NF-kB//I-kB, BID//Bcl-2, Bcl-X<sub>L</sub>//BID, Bcl-2//BID, FADD//caspase-9, BID//Bcl-X<sub>L</sub>, Bcl-X<sub>L</sub>//Hrk, Bcl-2//Hrk, TRADD//caspase-9, BID//A1/Bfl-1, Bcl-X<sub>L</sub>//BIM, Bcl-2//BIM, Apaf-1//caspase-9, Bcl-X<sub>L</sub>//Noxa, Bcl-2//Noxa, Bcl-X<sub>L</sub>//Bmf, Bcl-2//Bmf, Bcl-X<sub>L</sub>//Puma, Bcl-2//Puma, Bcl-X<sub>L</sub>//Bcl-G, 20 Bcl-2//Bcl-G, Bcl-X<sub>L</sub>//NIP3, Bcl-2//NIP3, Bcl-X<sub>L</sub>//Nix, and Bcl-2//Nix.

4. The method of claim 2 wherein said one or more intracellular complexes are selected from the group consisting of 14-3-3//BAD, Bcl-2//BAD, 14-3-3//BID, BAX//Bcl-2, Bcl-2//BIK, BID//Bcl-2, Bcl-2//BID, Bcl-2//Hrk, Bcl-2//BIM, Bcl-2//Noxa, Bcl-2//Bmf, Bcl-2//Puma, Bcl-25 2//Bcl-G, Bcl-2//NIP3, and Bcl-2//Nix.

5. The method of claim 4 wherein said disease is a cancer.

6. The method of claim 2 wherein said one or more intracellular complexes is NF-kB//I-kB.

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7. The method of claim 6 wherein said disease is an inflammatory condition.

8. The method of claim 2 wherein said one or more intracellular complexes are selected from the group consisting of Her1//Shc, Grb2//Sos, Her1//Grb7, Her1//RasGAP, Grb2//Shc, 35 Her2//Shc, Her3//PI3K, Her3//Shc, Her3//Grb7, YAP//Her4, IGF-1R//PI3K, IGF-1R//Shc, IGFR//IRS1, VEGFR//Shc, VEGFR//PI3K, VEGFR//Src, VEGFR//FRS2, PDGFRa//Crk,

PDGFR//Grb2, PDGFR//Grb7, PDGFR//Nck; PDGFR//Shc, DGFR//STAT5, PDGFRa//Crk, PDGFRb//GAP, PDGFR//Grb2, PDGFR//Grb7, PDGFR//Nck; PDGFR//Shc, PDGFR//Shp2, PDGFR//RasGAP, PDGFR//STAT5, PDGFRb//GAP, PDGFR//Grb2, PDGFR//Grb7, PDGFR//Nck, PDGFR//Shc, PDGFR//Shp2, PDGFR//RasGAP, PDGFR//STAT5, Kit//Shp-1, 5 Kit//PI3K, Kit//Grb2, Kit//CRKL, FGFR//PLCg1, FGFR//Crk, FGFR//FRS2, GFR//Shp2, FGFR//Shb, Trk//p75NTR, and Trk//PI3K.

9. The method of claim 8 wherein said disease is a cancer.

10 10. The method of claim 2 wherein said one or more intracellular complexes are selected from the group consisting of Her1//Shc, Grb2//Sos, Her1//Grb7, Her1//RasGAP, Grb2//Shc, Her2//Shc, Her3//PI3K, Her3//Shc, and Her3//Grb7.

11. The method of claim 10 wherein said disease is a cancer.

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12. The method of claim 11 wherein said cancer is breast cancer, ovarian cancer, colorectal cancer, or prostate cancer.

13. The method of claim 2 wherein said disease is a cancer and wherein said one or more 20 intracellular complexes are selected from the group consisting of IGF-1R//PI3K, IGF-1R//Shc, and IGFR//IRS1.

14. The method of claim 2 wherein said disease is a cancer and wherein said one or more intracellular complexes are selected from the group consisting of VEGFR//Shc, VEGFR//PI3K, 25 VEGFR//Src, and VEGFR//FRS2.

15. The method of claim 2 wherein said disease is a cancer and wherein said one or more intracellular complexes are selected from the group consisting of PDGFRa//Crk, PDGFR//Grb2, PDGFR//Grb7, PDGFR//Nck; PDGFR//Shc, PDGFR//STAT5, PDGFRa//Crk, PDGFRb//GAP, 30 PDGFR//Grb2, PDGFR//Grb7, PDGFR//Nck; PDGFR//Shc, PDGFR//Shp2, PDGFR//RasGAP, PDGFR//STAT5, PDGFRb//GAP, PDGFR//Grb2, PDGFR//Grb7, PDGFR//Nck, PDGFR//Shc, PDGFR//Shp2, PDGFR//RasGAP, and PDGFR//STAT5.

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16. The method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 wherein each of said one or more intracellular complexes are determined by the steps of:
- providing for each of said one or more intracellular complexes a reagent pair comprising  
5 a cleaving probe having a cleavage-inducing moiety with an effective proximity, and one or more binding compounds each having one or more molecular tags attached thereto by a cleavable linkage, the molecular tags of different binding compounds having different separation characteristics;
- 10 mixing the cleaving probe and the one or more binding compounds for each of said one or more intracellular complexes with said patient sample such that the cleaving probe and the one or more binding compounds specifically bind to their respective intracellular complexes and the cleavable linkages of the one or more binding compounds are within the effective proximity of the cleavage-inducing moiety so that molecular tags are released; and
- 15 separating and identifying the released molecular tags to determine the presence or absence or the amount of said one or more intracellular complexes in said patient sample.
17. A method of determining an apoptotic status of cells in a sample, the method comprising the step of simultaneously measuring amounts of at least one complex selected from the group consisting of a complex comprising a Bcl-2 protein and a BH3-only protein and a complex  
20 comprising a 14-3-3 protein and a BAD protein.
18. The method of claim 17 further including simultaneously measuring free NF-kB proteins and free I-kB proteins, and wherein said group further consist of a complex comprising an NF-kB protein and an I-kB protein.  
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19. The method of claim 18 wherein said group further consists of a homodimer comprising a BAX protein.
20. The method according to claim 17, 18, or 19 wherein each of said one or more  
30 intracellular complexes are determined by the steps of:
- providing for each of said one or more intracellular complexes a reagent pair comprising a cleaving probe having a cleavage-inducing moiety with an effective proximity, and one or more binding compounds each having one or more molecular tags attached thereto by a cleavable linkage, the molecular tags of different binding compounds having different separation  
35 characteristics;

mixing the cleaving probe and the one or more binding compounds for each of said one or more intracellular complexes with said patient sample such that the cleaving probe and the one or more binding compounds specifically bind to their respective intracellular complexes and the cleavable linkages of the one or more binding compounds are within the effective proximity of the cleavage-inducing moiety so that molecular tags are released; and  
5 separating and identifying the released molecular tags to determine the presence or absence or the amount of said one or more intracellular complexes in said patient sample.

21. A method of determining a status of a cancer in a patient, the method comprising the step of simultaneously measuring in a sample from the patient amounts of at least one intracellular complex selected from the group consisting of a first complex comprising a Bcl-2 protein and a BH3-only protein and a second complex comprising a 14-3-3 protein and a BAD protein.  
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22. The method of claim 21 wherein said at least one intracellular complexes is determined by the steps of:  
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providing for each of said first and second intracellular complexes a reagent pair comprising a cleaving probe having a cleavage-inducing moiety with an effective proximity, and one or more binding compounds each having one or more molecular tags attached thereto by a cleavable linkage, the molecular tags of different binding compounds having different separation characteristics;  
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mixing the cleaving probe and the one or more binding compounds for each of said first and second intracellular complexes with said patient sample such that the cleaving probe and the one or more binding compounds specifically bind to their respective intracellular complexes and the cleavable linkages of the one or more binding compounds are within the effective proximity of the cleavage-inducing moiety so that molecular tags are released; and  
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separating and identifying the released molecular tags to determine the presence or absence or the amount of said first and second intracellular complexes in said patient sample.

23. A method of determining an apoptotic status of cells in a sample, the method comprising the step of simultaneously measuring amounts of at least one complex selected from the group consisting of a complex comprising an NF-kB protein and an I-kB protein and amounts of free NF-kB proteins and free I-kB proteins.  
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